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Arbuscular mycorrhizal fungi (AMF) communities associated with cowpea in two ecological site conditions in Senegal

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The objective of this study was to characterize the diversity of arbuscular mycorrhizal fungal (AMF) communities colonizing the roots of *Vigna unguiculata* (L.) plants cultivated in two different sites in Senegal. Roots of cowpea plants and soil samples were collected from two fields (Ngothie and Diokoul) in the rural community of Dya (Senegal). Microscopic observations of the stained roots indicated a high colonization rate in roots from Ngothie site as compared to those from Diokoul site. The partial small subunit of ribosomal DNA genes was amplified from the genomic DNA extracted from these roots by polymerase chain reaction (PCR) with the universal primer NS31 and a fungal-specific primer AML2. Nucleotide sequence analysis revealed that 22 sequences from Ngothie site and only four sequences from Diokoul site were close to those of known arbuscular mycorrhizal fungi. Also, 47.6% of the clones from Ngothie site and 89.47% from Diokoul site were not close to known AMF. A total of 15 operational taxonomic units (OUT) were identified. Phylogenetic analyses showed that these clones belonged to the genera *Glomus*, *Sclerocystis*, *Rhizophagus*, *Scutellospora*, *Gigaspora*, *Racocetra*, *Acaulospora* and *Redeckera*. The genus *Glomus* is the most represented with six OTU, representing 40% of all OTU.

Key words: Agriculture, Glomeromycota, *Vigna unguiculata*, diversity, soil origin.

INTRODUCTION

Cowpea (*Vigna unguiculata* L.) is a legume that plays an important role in the nutritional balance of the rural population in Sub Saharan Africa, particularly in western and eastern regions where it is mostly cultivated. This

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legume as source of protein, occupies an important place in diet and population food security (Ndema et al., 2010). Its leaves are also highly appreciated by livestock (Cissé and Hall, 2003). Dry grains and fresh leaves contain a high level of protein (23-25%), vitamins B and micronutrients such as iron, calcium and zinc (Cissé and Hall, 2003). In addition, another important beneficial role of this legume is its contribution to soil nitrogen status through symbiotic nitrogen fixation, thereby enhancing soil fertility and reducing the need of N-fertilizer application (Bado, 2002).

The average yield per hectare of cowpea is variable. It ranges from 50 to 550 Kg/ha in Africa and depends on climatic conditions, varieties, cropping system and the level of fertilizer used and pesticides (Cissé and Hall, 2003). These yields are considered low as compared to the potential yield of up to 1000 and 3000 Kg/ha for the Senegalese varieties Melakh and Murid, respectively (Cissé and Hall, 2003). The low yield observed is mainly due to low rainfall and soil nutrient (P and N) depletion.

In all legume plants, cowpea is able to form symbiotic association with microorganisms such as rhizobia and arbuscular mycorrhizal fungi (AMF). This last can improve plant growth under low soil fertility condition, confer tolerance to some pathogens, improve the water balance of plants and can influence soil aggregation (Dalpé, 2005; Marulanda et al., 2006; Rillig and Mummey, 2006). Currently, about 250 species of AMF are described in the world (Jansa et al., 2014). These symbiotic microorganisms have been formerly identified by the morphological characteristics of their spores although sporulation is under the control of several parameters such as environment, seasonal variation, host plant, genotype and AMF species (Sanders, 2004; Smith and Read, 2008). Thus, spore counting and identification based on morphological characterization do not adequately reflect the diversity of active AMF communities in plant roots (Schüßler et al., 2001). Nowadays, several DNA-based methods have been used successfully in studies of diversity and could help to overcome these constraints. Among them, there might be mentioned PCR, quantitative PCR, Sanger sequencing and 454 pyrosequencing methods (Chifflot et al., 2009; Buée et al., 2009 ; Stockinger et al., 2010; Rousk et al., 2010; Tedersoo et al., 2010; Orgiazzi et al., 2013).

Furthermore, many AMF species are ubiquitous, occurring worldwide in quite different terrestrial ecosystems (Öpik et al., 2006), while others appear to be restricted to specific ecosystems, land uses types, vegetations or climates (Oehl and Sieverding, 2004; Castillo et al., 2006).

In Africa, several studies have shown that native AM fungi mainly belong to Glomeraceae, Gigasporaceae and Acaulosporaceae families (Bâ et al., 1996; Kachkouch et al., 2012). Most of them showed the predominance of the genus *Glomus* in several ecosystems, particularly in Senegal (Diop et al., 1994; Manga et al., 2007; Ndoye et al., 2012), Burkina Faso (Bâ et al., 1996) and Morocco (Abbas et al., 2006; Bouamri et al., 2006). However, a study conducted in Benin showed an equal distribution between *Glomus* and *Scutellospora* species in a woodland of *Isoberlina doka* (Houngnandan et al., 2009). This AMF diversity described in Benin is low as compared to that found in the reserve of Bandia in Senegal (Sene et al., 2012) and those in the arid and semi-arid areas of Africa and elsewhere (Bâ et al., 1996; Mohammad et al., 2003; Bouamri et al., 2006; Ndoye et al., 2012; Kachkouch et al., 2012). In Senegal, molecular diversity studies of AMF performed on acacia trees showed only the presence of *Glomus* in their roots, suggesting a preferential mycorrhization of these trees because other genera have been isolated in their rhizosphere (Manga et al., 2007; Ndoye et al., 2012).

Some studies have shown that different ecological conditions can influence the AMF community in cultivated soil (Oehl et al., 2010). However, in Sub-Saharan African particularly in Senegal, little is known about the influence of ecological site conditions on AMF community composition.

The aim of this study was to analyze the AMF communities composition in cowpea cultivated under two different ecological site conditions in Senegal.

MATERIALS AND METHODS

Study sites

The study was conducted in the rural community of Dya, located in the region of Kaolack, in the center of Senegal. This zone belongs to the eco-geographical area of the “South groundnut basin” and is characterized mostly by flat terrain dotted with depressions, representing the old dry valleys. The climate is Soudano-Sahelian with 3 to 4 months of rainy season and 8-9 months of dry season during which the temperature varies between 25°C in January and 45°C in May. The annual rainfall varies between 500 to 900 mm. There are four types of soil in this zone: “Dior” soil or arenosoil (75% of area), “Dek-dior” soil or lixisoil (16% of area), “Dek” soil or vertisoil (8% of area), and saline soil (1% of area) (Merlier, 1972).

The study was conducted at two sites. The first site is located in the village of Ngothie (14°N 14' W, 16°N 12' W) and the second in the village of Diokoul (14°N 15’W, 16°N 11' W). In Ngothie site, the field has been installed on a Dek soil whereas in Diokoul site, the field is on a Dior-soil.

On both sites, woody stratum is composed of acacia trees dominated by *Faidherbia albida* whose density is greater on Ngothie site than on Diokoul site. The herbaceous stratum is the same except *Adropogon gayanus* encountered only on Ngothie site (in zone of depression).

Plant material and experimental design

V. unguiculata (L.) seeds used in this study belonged to the “Melakh” variety. This choice was justified by its fast growing cycle (early variety: 60 days) allowing escape of the drought and it’s appreciation by farmers. Seeds provided by ISRA (Institut Sénégalais

de Recherches Agricoles, Dakar, Senegal) were directly used in fields without any previous treatment as used in traditional farming system.

The two sites are separated by about 2 km. At Ngothie, the field is installed on a Dek soil. Each field was divided into five plots of 16 m^2 each. In each plot, there are seven lines of seven seed holes each, representing a total of 49 seed holes. The distance between the lines and between the seed holes is 60 cm. At sowing, two seeds were placed into each hole.

Soil sampling and characterization

Before sowing, in each plot, soil was sampled at three points on the horizon 0-40 cm and mixed to get a composite soil sample. Soil samples from the five plots were pooled to one composite soil sample of approximately 2 kg per field. The composite soil sample from each field was passed through a coarse sieve (2 mm mesh) to remove stones and fine roots.

Physicochemical characterization of composite soil samples was performed at LAMA (Laboratoire des Moyens Analytiques de l'Institut de Recherche pour le Développement, Dakar, Senegal). The total amount of carbon and nitrogen was quantified by using the combustion system Thermo-Finnigan Flash EA 1112 (Thermo-Finnigan, France). The colorimetric determination of total and available phosphorus was performed according to the method of Dabin (1965). Soil pH values were measured in 2 M KCl suspensions at a solid liquid ratio of 1:2, 5. Soil physical characteristics were determined according to the method of Gee and Bauder (1986) and exchangeable cations following the method of Thomas (1982).

Plant harvested and analyses

Root sampling and assessment of AM colonization

After pod maturity (55 days after sowing), 10 cowpea plants were randomly chosen in each experimental plot, representing a total of 50 plants per field. Fine roots of 10 pooled plants (5 replicates per field) were collected, gently washed and stored in alcohol 70°C. Before assessment of AM colonization, roots were washed, cleared in KOH (10%) and stained with 0.05% Trypan blue (Philips and Hayman, 1970). Roots were then cut into pieces of 1 cm and placed on slides for microscopic observation at 400x magnification (Abbas et al., 2006). The frequency and intensity of mycorrhization were assessed following the method of Trouvelot et al. (1986) on 50 pieces per sample (250 root pieces per field).

AM fungal spore extraction, enumeration and morpho-anatomical identification

AMF communities were studied by spore extraction from soil samples. Hence 100 g soil (dry weight) of each subsample (with three replicates) was wet-sieving, following the methodology proposed by Gerdemann and Nicholson (1963). Samples were centrifuged in a sucrose gradient (Walker et al., 1982). Depending on the color, size, solitary character or grouped spores, mode of attachment of the hyphae and specific attributes, the spores were grouped into morphotypes. The relative abundance of each morphotype and AMF spore density (total number of spores in 100 g of dry soil) were determined with a normal appearance under a compound microscope (40x).

For taxonomic identification, AMF spores were mounted onto slides in polyvinyl alcohol (PVA) (Omar et al., 1979) with or without Melzer reagent (Morton, 1988). Specimens were identified with the original description and reference isolates described by the International Culture Collection of Arbuscular and Vesicular-Mycorrhizal Fungi (http://invam.wvu.edu/the-fungi/species-descriptions). In most cases, identification of the samples was assessed by the observation of morphological features of spores obtained after sieving and decanting.

Molecular analyses of AMF colonizing cowpea roots

For molecular analysis, roots were stored in alcohol at 70°C prior to DNA extraction. Fine roots from each field were washed, cut into pieces and pooled to get a composite root sample. DNA was extracted from 80 mg of the composite root sample (per field) using Fast DNA SPIN Kit following the manufacturer's recommendations. The DNA extraction (from 80 mg of roots) was repeated three times for each composite sample. The DNAs extracted from the three replicates were mixed in equimolar quantities to get a composite sample by taking into account the concentration.

The partial small subunit (SSU) region of the nuclear rDNA was used as a target region for the PCR experiment. DNA was amplified in a 25 µl reaction volume containing 5 µl of reaction buffer 5X ($MgCl_2$ 1.5 mM), 1 U of GoTaq polymerase, 200 µM dNTPs, 0.5 µl of BSA (200 ng/µl), 0.5 µl of each primer (20 µM), 14.05 µl of sterile water and 2 µl DNA template. The DNA amplification was performed using the universal eukaryotic primer NS31 (5'-TTG GAG GGC AAG TCT GGT GGC-3', Simon et al., 1992) and a fungal primer AML2 (5'-GAA CCC AAA CAC TTT GGT TTC C-3', Lee et al., 2008), designed to target as specifically as possible the *Glomeromycota*. The PCR was carried out as follows: an initial denaturation at 94°C for 3 min followed by 30 cycles at 94°C for 30 s, 58°C for 1 min, 72°C for 1 min 20 s, followed by a final extension period at 72°C for 10 min. PCR products were analyzed by electrophoresis using a 1% w/v agarose gel (Sigma, France) in a Tris-Acetate-EDTA buffer with a size standard (Eurogenic Smart Ladder). PCR products were then purified with the PCR purification kit (Invitrogen) according to the manufacturer's instructions. The purified PCR products were cloned into ultra-competent cells XL2 blue using PGEM-T Easy Vector (Promega/Catalys, Wallisellen, Switzerland) following the manufacturer's instructions. Twenty four Putative positive clones were selected and amplified using M13 forward and M13 reverse primers. Purified PCR products were then sequenced with the universal primers M13 F/M13 R (Genoscreen, France).

The sequences of clones were corrected using the Chromas software and compared by BLAST (Basic Local Alignment Search Tool) with those deposited in the NCBI (National Center for Biotechnology Information) data bank. All sequences obtained from this study were aligned using BioEdit software along with reference sequences from GenBank. Corrected sequences were submitted to GenBank and were allocated with accession numbers (KC588975 to KC589000). The clones from supprimer site of Ngothie site were called DEKRA and those from Diokoul site, DIORRA. The maximum likelihood (ML) tree was constructed using PHYML. The Kimura 2-parameter model with 100 bootstraps was used. The phylogenetic tree was computed using *Mortierella polycephala* (accession number X89436) and *Endogone pisiformis* (accession X58724) sequences as outgroups. Sequence phylotypes (called OUT in the text) were defined as separated monophyletic groups in the phylogenetic tree (Hijri et al., 2006).

Statistical analysis

Species richness, Shannon and Wiener diversity index (H'),

Table 1. Physicochemical characteristics of soils from the two study sites.

Soil characteristics	Ngothie Site (Dek soil)	Diokoul Site (Dior soil)
Clay (%)	4.4	1.3
Silt (%)	19.8	13
Fine sand (%)	50.6	29.2
Coarse sand (%)	14.1	55.7
Nitrogen Kjeldahl (%)	574	114
$N(NO_3)$ mg/kg	9.9	1.4
$N(NH_4)$ mg/kg	14.74	6.77
Total N (%)	0.055	0.022
Total C (%)	0.55	0.22
C/N	10	10
Total P (mg/kg)	139	51
Soluble P (mg/kg)	15	8
$pH(H_2O)$	6.1	5.8
pH (KCl)	5.5	4.8

(E) and Simpson dominance index (D) were calculated for each field in order to assess the diversity of AMF spores. The Shannon and Wiener diversity index was calculated using the following equation: $H' = -\sum p_i (\ln p_i)$ where *pi* represents the proportion of individuals found in the *i*th species, estimated as *ni /N*, *ni,* the number of individuals in the *i*th species and *N,* the total number of individuals. For a better interpretation of the results, the inverse of the Simpson dominance index was calculated as follows: $1\text{-}D = 1\text{-}\sum [ni (ni\text{-}1)/N (N\text{-}1)]$, where ni represents the number of the ith type and N the number of individuals in the population. The species distribution was determined by calculating the evenness using the following formula: $E = H'/H_{max}$ where *Hmax* is the maximum diversity and is calculated by ln *S*, where *S* is the number of species recorded.

Percentage of root colonization rate was arcsine transformed prior to analysis. Data were subjected to a one-way analysis of variance (ANOVA) using the XLSTAT software version 2010. Mean values were compared using the Student-Newman-Keuls range test ($P<0.05$) in order to determine the effects of site on these parameters.

Data on AMF spore density and relative abundance without any prior processing were also subjected to a one way analysis of variance using the same software. Mean values were compared using the Student-Newman-Keuls range test ($P < 0.05$). This analysis was performed to determine the effect of site in the spore density.

RESULTS

Physicochemical characteristics of soils

Results of soil physicochemical analyses are presented in Table 1. Data showed that both soils were acidic. The soil from Diokoul site (Dior) was slightly more acidic than those from Ngothie site (Dek). The highest percentage of organic and all mineral nutrient contents were observed in soil from Ngothie site. The percentages of fine sand and clay were higher in soil from Ngothie site whereas that of coarse sand was greater in soil from Diokoul site.

AMF spore density, species richness and diversity measurement of AMF spores communities

The AMF spore density varied between the two sites (Figure 1). The AMF spore number was significantly ($P=0.02$) greater in soil from Ngothie (636 spores/100 g of soil) than in soil from Diokoul (293 spores/100 g of soil).

In both sites, the highest spore density was recorded for *Entrophospora* sp. with 254 and 197 spores per 100 of dry soil for Ngothie and Diokoul sites, respectively. However, the lowest spore number was obtained with *Glomus* (7 spores/100 g of dry soil) in soil from Ngothie, and with *Scutellospora* in soil from Diokoul site (2.67 spores/100 g of dry soil).

In both sites, a total of ten AMF morphotypes belonging to seven genera were recorded and *Glomus* represented the most diverse genus with three morphotypes (Table 2). The number of AMF morphotypes (Table 2) showed that the soil from Ngothie site displayed 9 AMF morphotypes and that from Diokoul site, 8 AMF morphotypes belonging to seven genera (*Glomus*, *Scutellospora*, *Gigaspora, Entrophospora*, *Acaulospora, Sclerocystis* and *Rhizophagus*). From the ten AMF morphotypes, seven were common to the two fields whereas *Glomus* sp1 and *Rhizophagus* sp. were isolated only in Ngothie site and *Sclerocystis* sp. only in Diokoul site.

Three diversity indexes were calculated to characterize the communities of AMF in both soils. Shannon-Weiner (H') and Simpson's indices showed variation between the two sites and were higher in Ngothie site than in Diokoul site. However, the species evenness was greater in Diokoul site than in Ngothie site (Table 3).

Intensity and frequency of mycorhization

Before exploring the diversity of AMF in the roots, mycorrhizal parameters of cowpea roots (intensity and frequency of mycorrhization) were first determined. Results showed that the cowpea root mycorrhizal colonization was significantly different between the two sites ($P<0.05$). The higher cowpea root mycorrhizal colonization was recorded in the field installed in Ngothie site (100 and 25% for frequency and intensity, respectively) compared to that of the field located in Diokoul site (60% and 5% for frequency and intensity, respectively) (Figure 2).

Community composition of AMF colonized roots of cowpea grown in both soils

A total of 42 clones obtained from root samples from Ngothie site and 38 clones for samples from the Diokoul

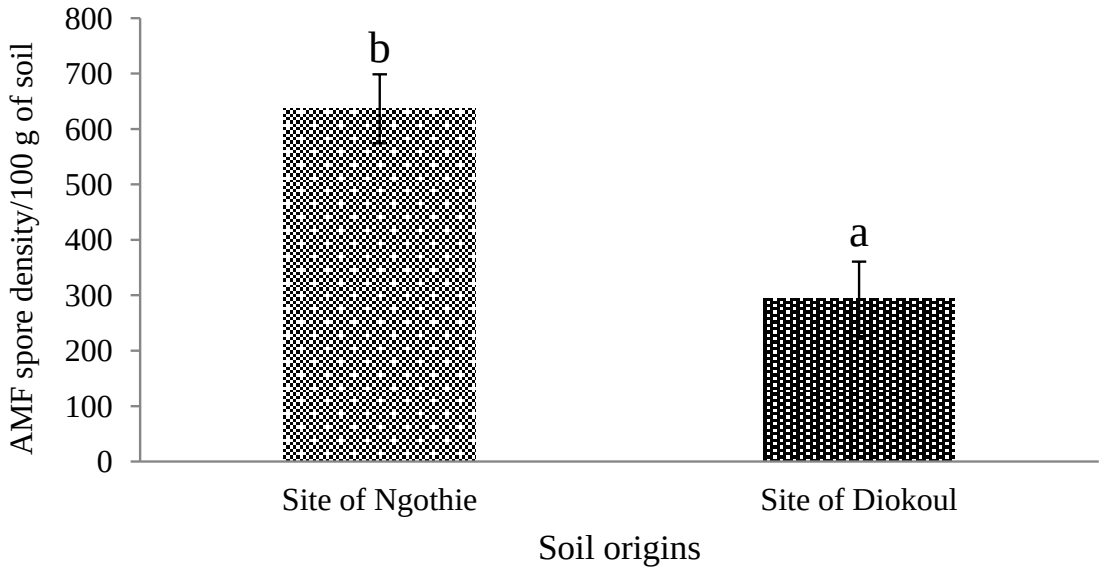

Figure 1. AMF spore density in rhizospheric soils of two cowpea growing fields. Bars with the same letter are not significantly different according to the Student-Newman-Keuls test ($P < 0.05$).

Table 2. Average density of each arbuscular mycorrhizal fungi (AMF) species or morphotype in 100 g of dry soil from the two study sites.

Parameter	Ngothie site (Dek soil)	Diokoul site (Dior soil)
Scutellospora gregaria	8.33	6.67
Scutellospora heterogama	51.33	2.67
Gigaspora spp.	46.00	31.66
Acaulospora longula	72	28.67
Entrosphospora spp.	254.33	198.00
Sclerocystis sp.	0.00	6.00
Glomus coronatum	13.67	4.3
Glomus sp1	7.00	0.00
Glomus spp.	167.00	15.33
Rhizophagus sp.	17.00	0.00
Number of AMF morphotypes	9	8

Gigaspora spp., *Entrophospora* spp. and *Glomus* spp. are considered as morphotypes because it would include more species. *Rhizophagus* sp, *Sclerocystis* sp and *Glomus* sp1 have not been identified and we gave them respectively the names *Rhizophagus* sp. and *Glomus* sp. *Sclerocystis* sp1.

Table 3. Diversity measurements of AMF communities from spores in soils from the two fields.

Parameter	Equations	Ngothie site (Dek soil)	Diokoul site (Dior soil)
Shannon Weiner index	$H' = -\sum p_i (\ln p_i)$	0.713	0.503
Simpson index of dominance (D)	$1-D = 1-\sum [ni (ni-1)/N (N-1)]$	0.746	0.719
Evenness (E)	H'/H_{max}	0.748	0.757

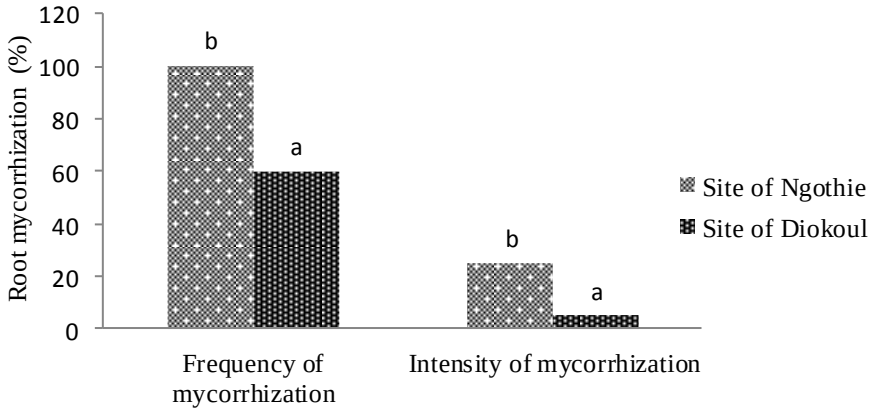

Figure 2. Mycorrhizal frequency and intensity of cowpea roots in the two fields. For each parameter, bars with the same letter are not significantly different according to the Student-Newman-Keuls test ($P < 0.05$).

site were sequenced. The number of clones identified as sequences of arbuscular mycorrhizal fungi in root samples were 22 for the soil from Ngothie site and only 4 for the soil from Diokoul site with a similarity percent of 52.38 and 10.52%, respectively (Figure 3). The number of clones identified as *Mucuna pruriens*, a *Fabaceae* close to the studied plant (*V. unguiculata L.*) is 18 and 30, respectively for the roots from Ngothie site and Diokoul site, respectively with a percentage of similarity comprised between 99 and 100%. Two and four clones were identified as others, respectively for soil from Ngothie site and that from Diokoul site.

The phylogenetic tree was built from alignment of the partial SSU fragments of about 560 nucleotides generated by NS31 and AML2. Different clades of the tree have allowed distinguishing 15 AMF OTU (operational taxonomic unit) belonging to seven known genera: *Scutellospora*, *Gigaspora*, *Redeckera*, *Acaulospora*, *Glomus*, *Sclerocystis* and *Rhizophagus* (Figure 3). The OUT *Gigaspora* (KC588982 and KC588994) formed a separate group in the family *Gigasporaceae* but their position was not well-defined. The different genera identified belonged to four families: *Gigasporaceae* (*Scutellospora*, *Gigaspora*, and *Racocetra*), *Diversisporaceae* (*Redeckera*), *Acaulosporaceae* (*Acaulopora*) and *Glomeraceae* (*Glomus*, *Sclerocystis, Rhizophagus*). These four families belonged to two orders: Diversisporales (*Gigasporaceae*, *Acaulosporaceae* and *Diversisporaceae*) and Glomerales (*Glomeraceae*). The *Glomeraceae* and the *Gigasporaceae* are the most diverse families as compared to *Acaulosporaceae* and *Diversisporaceae,* each represented by a single genus. Also, the results indicated that the sequences of the AMF found in the roots of cowpea were different from known AMF species listed in GenBank database.

Shannon-Weiner index (H') based on DNA sequences of AMF revealed differences between both root origins. This index was relatively higher in samples from Ngothie site (1.06) compared to those from Diokoul site (0.45).

DISCUSSION

The root DNA amplification and sequencing with specific primers revealed the presence of several AMF species in the roots of cowpea. These AMF species belonged to eight genera, which are *Gigaspora*, *Scutellospora*, *Acaulospora*, *Glomus*, *Sclerocystis*, *Redeckera*, *Rhizophagus* (Schüßler and Walker, 2010) and *Racocetra* (Oehl et al., 2008). These results confirmed the ability of the primer AML2 to amplify several groups of AMF and indicated a wide diversity of AMF in roots of cowpea (Lee et al., 2008). However, these findings were opposed to those of Manga et al. (2007) and Ndoye et al. (2012) who found only species of the genus *Glomus* in rhizosphere of *Acacia senegal* in the semi-arid zone of Bambey and Dahra (Senegal), respectively. Nevertheless, Ngonkeu (2009) using the same fungal primers as the previous authors (LR1, FLR4), had already found some Gigasporaceae and Acaulosporaceae species in the roots of corn in Cameroon. These results might be explained by a selective mycorrhization (Helgason et al., 2002) in acacia trees because other species of fungi were found in their root zone particularly in *Acacia senegal* (Ndoye et al., 2012). The genus *Glomus* is the most diverse in our study with six species, representing 40% of all the species found in roots of cowpea. The predominance of this genus in most ecosystems like Senegal (Manga et al., 2007; Sene et al., 2012 ; Ndoye et al., 2012), China (Zhao and Zhao, 2007; Wang et al., 2008), Burkina Faso

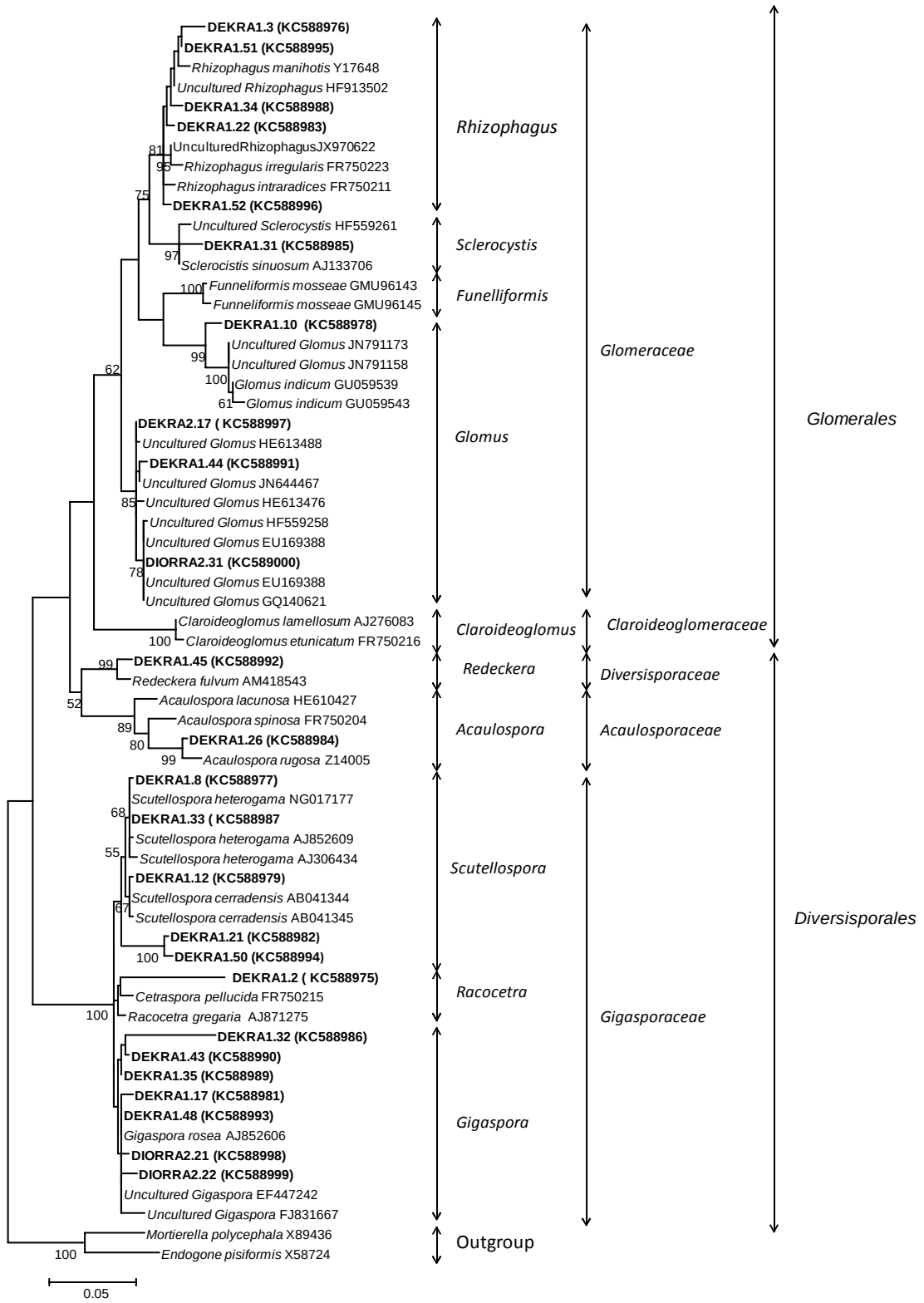

Figure 3. Maximum likelihood tree showing all sequences identified in cowpea roots. Sequences obtained in the present study are shown in bold. Bootstrap support values for branches were estimated from 1.00 replicates. The corresponding accession numbers ranging from KC588975 to KC599000 were given by Genbank. DEKRA means clones from soil DEK (Ngothie site) and DIORRA clones from DIOR soil (Diokoul site).

(Bâ et al., 1996) and central Europe (Oehl et al., 2003) suggests a better adaptation of the genus to adverse conditions such as warm, cold, drought, salinity and other environmental stress (Blaszkowski et al., 2002). The dominance of *Glomus* in these agro-ecosystems might also be related to their ability to form anastomoses after hyphae break. Indeed, as compared to Gigasporaceae, the Glomeraceae form more easily anastomoses between hyphae of the mycelium and may have the ability to restore an interconnected network after mechanical disruption of the hyphae (de la Providencia et al., 2005).

Furthermore, our results indicated that the clones close to uncultured *Gigaspora* EF447242 is the most frequent species encountered in the roots of cowpea with eight sequences out of the twenty-two, representing 27.27% of the all clones. In other cases, by studying the seasonal and spatial trends of fungal populations, Merryweather and Fitter (1998) reported the dominance of *Scutellospora dipurpurescens* in the roots of *Hyacinthoides non-scripta* in autumn and winter while *Acaulospora* and *Glomus* were dominant in spring. Dominance of *S. dipurpurescens* will be explained by its positive correlation with shoot P content and carbohydrates availability for the fungus. This important contribution to the absorption of P in Gigasporaceae at the expense of the other groups of fungi was noted by Maherali and Klironomos (2007). This is due to their ability to produce root outside a large amount of hyphae able to explore a large volume of soil (Hart and Reader, 2002; Maherali and Klironomos, 2007).

Also, this strong presence of the genus *Gigaspora* in the roots of cowpea could be explained on one hand by its strong presence in the soil and on the other hand by the composition of root exudates. Indeed, Redmond et al. (1986) and D'Arcy-Lameta (1989) demonstrated that flavonoids are produced exclusively by leguminous plants.

These compounds have often been associated with the attraction of *Gigaspora margarita* and *Glomus* spp. spore germ tubes by plant roots (Tsai and Phillips, 1991; Bécard et al., 1992). This can promote root colonization by these AMF, promoting spore germination.

As demonstrated by Lee et al. (2008), the results have also shown that the primer AML2 was not specific to *Glomeromycota*. Based on the fact, coupled with NS31, it has amplified DNA of other organisms and DNA of plant, as attested by the blast result (18 clones in soil from Ngothie site and 30 in soil from Diokoul site were identified as *Mucuna pruriens* (tribe of Phaseoleae). Therefore, there is a very high probability of amplifying non-specific DNA products during PCR, especially with environmental material from field soils (Clapp et al., 2002). However, other primers such as AML1 (Helgason et al., 1998) were not exclusive of *Glomeromycota*, amplifying other taxa like parasitic fungi (Redecker, 2000; Lee et al., 2003).

Our results on the mycorhization rate, spore density and Shannon diversity indices might be explained by soil properties from the two sites. It is long recognized that very high or very low phosphorus levels may reduce mycorrhization and/or sporulation. The lower rate of mycorrhization and the low sporulation in soil from Diokoul site could be due to low soil P level in comparison with Ngothie soil. These results are in accordance with those of Pierart (2012) and Lagrange (2009) which showed that the very low P level inhibits the mycorrhization.

Apart from soil phosphorus, the difference noted in the diversity of AMF at the two study sites might be due to the surrounding flora. This flora is composed of an herbaceous stratum and shrub stratum. The shrub stratum is mainly composed of *Faidherbia albida* whose density is greater on the site of Ngothie (in Dek soil) than in that of Diokoul (in Dior soil). These acacia trees are usually associated with a great diversity of arbuscular mycorrhizal fungi (Belay et al., 2013) that might allow active AMF dispersal due to the growth speed of the root system (Diop et al., 1994).

Also, a slight modification of species composition can be noted with the presence of *Andropogon gayanus* only on the site of Ngothie. This difference in floristic composition might influence the diversity of arbuscular mycorrizal fungi (Diallo et al., 1998).

Conclusions

The results of this study indicate that cowpea plant is associated with a range of AMF belonging to eight genera and four families. Also, the results showed that a species of *Gigaspora* close to uncultured *Gigaspora* EF447242 was largely dominant in roots of cowpea (27% of clones in Ngothie site and 50% in Diokoul site). However, the family of Glomeraceae was the most represented and the most diverse with 53.3% of AMF species. The results suggested that ecological site conditions may influence AMF diversity and colonization rate *in planta*.

Conflict of interests

The authors did not declare any conflict of interest.

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